

# The cardiovascular implication of single nucleotide polymorphisms of chromosome 9p21 locus among Arab population

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**Background:** Based on several reports including genome-wide association studies, genetic variability has been linked with higher (nearly half) susceptibility toward coronary artery disease (CAD). We aimed to evaluate the association of chromosome 9p21 single nucleotide polymorphisms (SNPs): rs2383207, rs10757278, and rs10757274 with the risk and severity of CAD among Arab population. **Materials and Methods:** A prospective observational case-control study was conducted between 2011 and 2012, in which 236 patients with CAD were recruited from the Heart Hospital in Qatar. Patients were categorized according to their coronary angiographic findings. Also, 152 healthy volunteers were studied to determine if SNPs are associated with risk of CAD. All subjects were genotyped for SNPs (rs2383207, rs2383206, rs10757274 and rs10757278) using allele-specific real-time polymerase chain reaction. **Results:** Patients with CAD had a mean age of  $57 \pm 10$ ; of them 77% were males, 54% diabetics, and 25% had family history of CAD. All SNPs were in Hardy-Weinberg equilibrium except rs2383206, with call rate >97%. After adjusting for age, sex and body mass index, the carriers of GG genotype for rs2383207 have increased the risk of having CAD with odds ratio (OR) of 1.52 (95% confidence interval [CI] = 1.01-2.961,  $P = 0.046$ ). Also, rs2383207 contributed to CAD severity with adjusted OR 1.80 (95% CI = 1.04-3.12,  $P = 0.035$ ) based on the dominant genetic model. The other SNPs (rs10757274 and rs10757278) showed no significant association with the risk of CAD or its severity. **Conclusion:** Among Arab population in Qatar, only G allele of rs2483207 SNP is significantly associated with risk of CAD and its severity.

**Key words:** Chromosome 9p21, coronary artery disease, Qatar, single nucleotide polymorphisms

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## INTRODUCTION

Despite advances in diagnosis and treatment, coronary artery disease (CAD) still leads the causes of death worldwide.<sup>[1]</sup> Several reports including genome-wide association studies (GWAS), have reported that nearly half of the case with genetic polymorphism are susceptibility to the development of CAD.<sup>[1-9]</sup> Gene variants are believed to have a correlation with the traditional risk factors for myocardial infarction (MI).<sup>[2,3]</sup> In addition, genetic factors have been shown to be particularly important for increasing the risk of early onset MI.<sup>[4,5]</sup> Therefore, a better understanding of the molecular mechanisms and genetic pattern could allow early detection of individuals who are at high risk of CAD, which facilitate targeted preventive therapies.

The association between polymorphism and development of CAD has been established by several

GWAS including multinational cohort analysis.<sup>[6-8]</sup> Recent studies have identified the single nucleotide polymorphisms (SNPs; rs2383207, rs10757278, and rs10757274) on chromosome 9p21 that are independently associated with CAD in different populations.<sup>[6-11]</sup>

The 9p21 genome region is a complex region near the protein-coding genes CDKN2A (cyclin-dependent kinase inhibitor 2A) and CDKN2B (cyclin-dependent kinase inhibitor 2B) respectively. The potential relationship between 9p21 and atherosclerosis may be interrelated to the antiproliferative action of the cyclin-dependent kinase inhibitors.<sup>[12-14]</sup> Functional studies have demonstrated previously enhanced expression of the noncoding RNA, antisense noncoding RNA in the INK4 locus [ANRIL], in 9p21 carriers and its role in the atherosclerosis process. In addition, deletion of the orthologous 70 kb noncoding interval on mouse chromosome 4 has provided direct evidence that the

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9p21 CAD risk interval has a direct role in the regulation of CDKN2A/B expression and affects CAD progression by altering the dynamics of vascular cell proliferation.<sup>[15]</sup>

Through using genome-wide association scanning, they showed that a common genetic variant on chromosome 9p21 (rs2383207 and rs10757278) associates with MI and is located nearby the tumor suppressor genes CDKN2A and CDKN2B, these two genes play a role in regulating cell proliferation, aging and apoptosis, which are the characteristic of atherogenesis, the fundamental cause of MI and CAD.<sup>[7]</sup> A gene ANXA1 is located on chromosome 9 it is a glucocorticoid anti-inflammatory messenger that is expressed in the microvascular endothelial cells of the brain.<sup>[16]</sup> Another gene located on 9q21 is FXN gene that is responsible for encoding a protein called frataxin. Mutation of this gene leads to Friedreich ataxi.<sup>[17]</sup>

To the best of our knowledge and belief, there is a paucity of information regarding genetic polymorphism and its relationship with the risk of CAD in Qatar. Herein, we aim to conduct a case-control association study to evaluate the association of chromosome 9p21 SNPs (rs2383207, rs10757278, and rs10757274) with CAD among Arab population residing in Qatar. We are also intended to establish the reliability of these locus-specific contributions to the CAD development in Arab population.

## MATERIALS AND METHODS

A prospective case-control study was conducted on unrelated Arab participants in Qatar. Male and female patients of age between 30 and 85 years with the clinical diagnosis of CAD were consecutively enrolled from the Cardiology Department, Heart Hospital, Qatar. Definitions of the patient outcome parameters as well as the diagnosis of CAD were made according to the American College of Cardiology Clinical Data Standards.<sup>[18]</sup> All patients underwent coronary angiograms on the index admission. The degree of atherosclerotic lesions and number of coronary vessels affected were assessed. Patients were categorized according to their coronary angiographic findings that is, Group I: With nonsignificant atherosclerotic lesion (<50% stenosis) and Group II: Atherosclerotic lesion causing luminal stenosis  $\geq$ 50% (significant lesions).<sup>[19]</sup> Quantitative angiographic assessment was performed using the Gensini and Sullivan extent systems and all coronary angiography evaluations were performed without knowing the genotype status.<sup>[15]</sup>

A consent form was obtained from each participant. Also, 152 healthy volunteers from centers other than Hamad Medical Corporation (HMC) were studied to determine if SNPs are associated with increased risk of CAD. Upon their

agreement, volunteers underwent a history and clinical examination including anthropometric measurements and vital signs and to exclude any cardiac problems.

### Exclusion criteria

Coronary artery disease patients with cancer, autoimmune disorders and any other diseases that might cross react with the variables under investigation were excluded.

Subjects with Type 2 diabetes mellitus (DM) were diagnosed according to the World Health Organization (WHO) criteria.<sup>[20]</sup> National Cholesterol Education Program Adult Treatment Panel III criteria was used to diagnose dyslipidemia.<sup>[21]</sup> The cut-off points used for body mass index (BMI) was according to the "WHO" criteria.<sup>[22]</sup> BMI was calculated by dividing the weight (Kg) with the square of height (m<sup>2</sup>). Hypertension is defined as a systolic and/or diastolic blood pressure of  $\geq$ 140 and/or  $\geq$ 90 mmHg or patient had a significant history of hypertension. A subanalysis was also performed to compare CAD patients with and without DM.

### Biochemical assays

A 10 ml of peripheral blood was collected from each participating subject. A volume of 5 ml was used for DNA extraction, and the remaining 5 ml was used for biochemical analysis including glucose, lipids, and cardiac markers. All biochemical assays were done at clinical chemistry laboratory at HMC, Qatar. Genetic analysis was performed in biomedical labs at Qatar University.

### Genotyping

Three SNPs (rs10757274, rs10757278, and rs2383207) located in chromosomal locus 9p21 were selected for the present study; the selection of these SNPs was based upon previous reports.<sup>[6-11]</sup>

The genotyping was done as previously published.<sup>[23]</sup> In brief, DNA was extracted from whole blood samples of all participating subjects, using the EZ1 DNA blood Kit from Qiagen Cat#951054 (Germany), according to the manufacturer protocol. Measurements of DNA concentration and purity were done using nanodrop spectrophotometer. All SNPs were genotyped by allele-specific PCR, in which primers were designed to specifically amplify the reference alleles. Polymorphisms of these SNPs were carried out by the 5' nuclease assay using TaqMan MGB probe by means of an ABI 7500 (Applied Biosystems, Foster City, CA). The primers and the probes of these SNPs were provided by the assay on demand TM service by Applied Biosystems. The 5' nuclease assay was performed using 20 ng of genomic DNA, 1x TaqMan (850 Lincoln Centre Drive, Foster City, CA) Universal PCR Master Mix (Applied Biosystems), and 1x primer/probe mix using the appropriate cycling conditions for amplifications according to manufacturer's instructions.

### Statistical analysis

Data were expressed as mean ± standard deviation, with 95% confidence interval (CI = 5-95%) for nonnormally distributed continuous data and by number and percentage for categorical data. Data were explored for outliers, skewness, and normality and were transformed if the normality assumption was violated.

Differences between continuous variables were analyzed by Student's *t*-test and ANOVA wherever applicable. Nonparametric Mann-Whitney and 2-independent samples *t*-tests were used accordingly for the analysis. Genotype distribution and allelic frequencies between the study groups were compared by constructing 2 × 2 contingency tables and  $\chi^2$  or Fischer exact test corrected for Bonferroni adjustment for the number of SNPs. The Hardy-Weinberg equilibrium was performed using the  $\chi^2$  test to assess genotype distribution in all study subjects. Three genetic models that is, dominant minor allele, recessive minor allele and additive models (additional copy of the risk alleles increases the response by an equal amount) were analyzed. Odds ratio (ORs) and 95% CI and corresponding *P* values were analyzed by logistic regression analysis and adjusted accordingly. ORs were computed using the minor allele homozygous as the reference group unless mentioned otherwise. A two-tailed *P* < 0.05 was considered as statistically significant. All statistical analyses were performed using the SPSS program for Windows (version 20 statistical software; Texas instruments, IL, USA) and Golden Helix' SNP and Variation Suite (SVS 7), Bozeman, MT, USA for genetic analysis.

## RESULTS

Patients with CAD (*n* = 236) had a mean age of 57 ± 10; of them, 77% were males, 54% diabetics, and 25% had family history of CAD. Subjects in the control group (*n* = 152) were 11 years younger, and 73% were males. Clinical features of the study population are shown in Table 1.

### Association between single nucleotide polymorphisms and coronary artery disease

#### Genotype distribution and minor allele frequency

Overall, the SNP genotypes and alleles frequencies were determined in 386 individuals. Among the study subjects, all examined polymorphisms were in Hardy-Weinberg equilibrium for rs10757278, rs10757274, and rs2383207 (*P* > 0.05) respectively. The call rate was above 85% for all studied SNPs. The frequency distribution of genotype was not significantly different for rs10757278, rs10757274, and rs2383207 between cases and controls (*P* = 0.543, *P* = 0.437 and *P* = 0.378, respectively) as shown in Figure 1. Age and sex showed no significant impact in patients with GG allele (*P* = 0.72).

### Association between alleles with risk of angiographic coronary artery disease lesion

The genetic association of the minor and major allele was assessed by the OR along with their 95% CI for all SNPs by logistic regression analysis. Interestingly, the G allele of rs2383207 increases the odds of angiographic lesions in coronary arteries among the study subjects by ≈1.5 folds (95% CI = [1.06-2.11]; *P* = 0.019); whereas, the A allele decreases this risk significantly [Table 2a]. No significant association was observed for the genetic variants rs10757278 and rs10757274 with the risk of having angiographic lesions among the

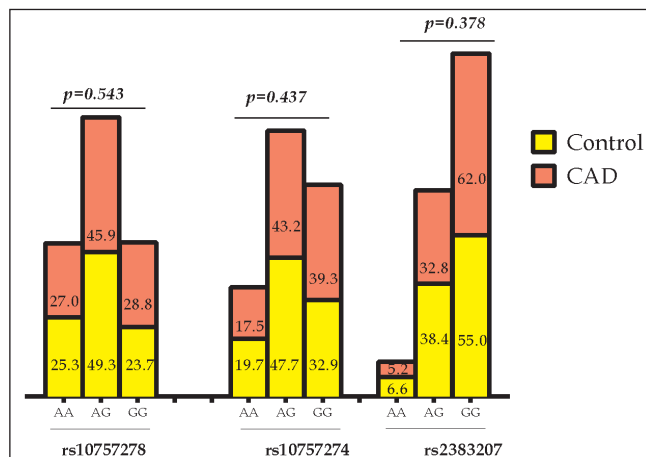


Figure 1: Genotype distribution of rs10757278, rs10757274 and rs2383207 among healthy controls and coronary artery disease patients

Table 1: Demographic data for healthy controls and CAD patients

Variables	CAD (n = 236)	Controls (n = 152)	<i>P</i>
Age (years)	56.57 (9.80)	44.77 (11.16)	0.000
Gender (%)			
Male	182.0 (77.12)	111.0 (73.03)	0.091
Female	54.0 (22.88)	42 (26.97)	
BMI (kg/m <sup>2</sup> )	31.64 (7.55)	32.57 (6.74)	0.702
Hypertension (%)	153.0 (64.83)	25.0 (16.45)	0.000
DM (%)	127.0 (53.81)	32.0 (21.05)	0.000
Smoker (%)	98.0 (41.53)	13.0 (8.55)	0.001
Family history (%)	58.0 (24.57)	9.0 (5.92)	0.021

CAD = Coronary artery disease; BMI = Body mass index; DM = Diabetes mellitus

Table 2a: Association between alleles with risk of CAD compared to healthy controls

SNP variant	OR for minor allele "A" and major allele "G"		<i>P</i>
	OR (95% CI) for A allele	OR (95% CI) for G allele	
rs10757278	0.86 (0.64-1.14)	1.16 (0.87-1.54)	0.304
rs10757274	0.83 (0.62-1.11)	1.21 (0.90-1/61)	0.204
rs2383207	0.66 (0.47-0.94)	1.49 (1.06-2.11)	0.019

ORs, 95% CI, and *P* value were evaluated by logistic regression analysis, adjusted for age, gender and BMI. Logistic regression analysis was used for the evaluation (OR and 95% CI) among CAD patients with healthy volunteers as a control group. Two-tailed *P* value is significant <0.05. CAD = Coronary artery disease; OR = Odds ratio; CI = Confidence interval; SNP = Single nucleotide polymorphism; BMI = Body mass index

study subjects. After adjustments of the CAD risk factors; the nonmodifiable CAD risk factors (age, gender) and the modifiable risk factors (smoking, hypertension, dyslipidemia, BMI and DM), the G allele increases the odds of having CAD by 3.38 folds (95% CI [1.54-7.41];  $P=0.002$ ) for rs10757274 and by 15.3 (95% CI [3.83-60.87];  $P<0.001$ ) for rs2383207 [Table 2b].

**Association between coronary artery disease and chromosome 9p21 based upon genetic models**

Using the dominant genetic model (GG vs. AG + AA), only carriers of GG genotype had the odds to have CAD by 1.5 folds (1.01-2.96),  $P=0.04$  for rs2383207 polymorphism, while carriers of AA alleles showed a significantly protected effect against the CAD risk (OR = 0.65 folds [0.44-0.98],  $P=0.04$ ). No significant association was observed for rs10757278, and rs10757274 polymorphism for AA carriers compared with the G allele (XG genotype) to have the risk of CAD under the recessive and dominant genetic models as shown in Table 3.

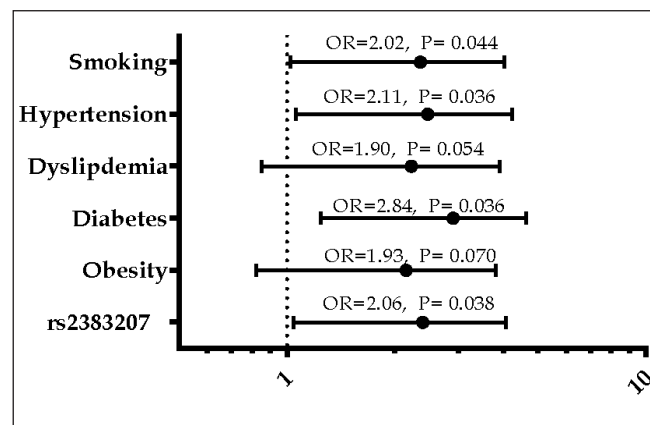
**Association between individual coronary artery disease risk factors phenotype and rs2383207 (genetic dominant model)**

The effect of GG carriers of rs2383207 with the individual CAD risks was assessed by logistic regression analysis as shown in Figure 2. Subjects having family history of CAD and GG carriers for rs2383207 had two-fold increased risk of CAD (OR = 2.06 95% CI [1.06-4.06]). In addition, diabetes (OR = 2.84, 95% CI, 1.24-4.63), hypertension (OR = 2.11, 95% CI, 1.24-4.23) and smoking (OR = 2.02, 95% CI, 1.24-4.63) significantly increased the risk of CAD among GG carrier patients, after adjusting for the other individual CAD risk factors.

**Association between single nucleotide polymorphisms and severity of cad lesions**

**Genotype distribution and minor allele frequency**

Figure 3 shows the genotype distribution among CAD patients based on the severity of stenosis. Of note, no significant difference was observed in genotype distribution and allelic frequency.



**Figure 2:** Odds ratio of the individual coronary artery disease (CAD) risk factors with rs2383207 (utilizing dominant model; GG/AA+AG) among the CAD patients, with healthy volunteers as a control

**The genetic association and the severity of angiographic lesions using genetic models**

Only the dominant genetic model (GG vs. AG + AA) showed that carrier of GG allele in CAD patients significantly increases the odds of the severity of stenosis (OR = 1.8 95%

**Table 2b: Association between alleles with risk of CAD compared to healthy controls**

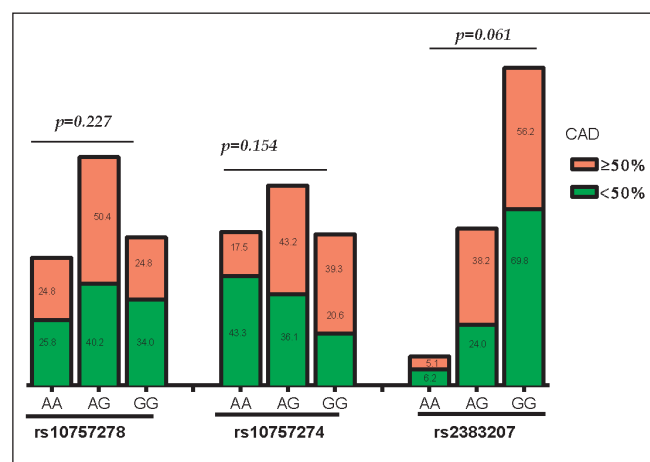
SNP variant	OR for minor allele "A" and major allele "G"			
	OR (95% CI) for G allele	P	Adjusted OR* (95% CI) for G allele	P
rs10757278	1.16 (0.87-1.54)	0.304	3.00 (0.812-11.08)	0.099
rs10757274	1.21 (0.90-1/61)	0.204	3.38 (1.54-7.41)	0.002
rs2383207	1.49 (1.06-2.11)	0.019	15.26 (3.83-60.87)	<0.001

Data were analyzed by logistic regression analysis, adjusted for nonmodifiable risk CAD factors were; age, and gender. Adjusted ORs was for the modifiable risk factors were; smoking, hypertension, dyslipidemia, BMI and diabetes. Logistic regression analysis was used for the evaluation (OR and 95% CI) among CAD patients with healthy volunteers as a control group. Two-tailed P value is significant <0.05. CAD = Coronary artery disease; OR = Odds ratio; CI = Confidence intervals; SNP = Single nucleotide polymorphism; BMI = Body mass index

**Table 3: The risks of CAD using different genetic model**

SNP variant	AOR (95% CI)	P
rs10757278		
AA/AG+GG	0.82 (0.52-1.30)	0.404
AA+AG/GG	0.75 (0.43-1.21)	0.324
GG/AG+AA	1.21 (0.77-1.91)	0.215
rs10757274		
AA/AG+GG	0.68 (0.41-1.17)	0.164
AA+AG/GG	0.86 (0.56-1.31)	0.474
GG/AG+AA	1.16 (0.76-1.77)	0.474
rs2383207		
AA/AG+GG	0.161 (0.16-1.12)	0.076
AA+AG/GG	0.65 (0.44-0.98)	0.046
GG/AG+AA	1.52 (1.01-2.961)	0.042

Data were analyzed by logistic regression analysis, adjusted for CAD risk factors; age, gender, smoking, hypertension, dyslipidemia, BMI and diabetes among CAD patients with healthy volunteers as a control group. Two-tailed P value is significant <0.05. SNP = Single nucleotide polymorphisms, CAD = Coronary artery disease, OR = Odds ratio, CI = Confidence interval, AOR = Adjusted odds ratio; BMI = Body mass index



**Figure 3:** Genotype distribution of rs10757278, rs10757274 and rs2383207 among coronary artery disease patients based upon the severity of stenosis by angiography



CI [1.04-3.12];  $P = 0.035$ ) and remained significant even after adjustment for rs2383207 polymorphism (OR = 1.74 95% CI [1.11-3.21],  $P = 0.03$ ). In contrast, the recessive genetic model (AA/AG + GG) showed no significant association with the severity of CAD [Table 4].

**Assessment of factors contributed to the severity of coronary artery disease lesion**

Regression analysis for the severe lesion with reference to nonsignificant lesion demonstrated that the following independent variables have a significant effect on the severity of stenosis. Therefore, smoking (OR = 2.02,  $P = 0.044$ ), diabetes (OR = 2.84,  $P = 0.036$ ), hypertension (OR = 2.11,  $P = 0.036$ ) and rs2383207 variant (OR = 2.06,  $P = 0.038$ ) were the most significant factors affecting the degree of stenosis [Figure 2].

**Association between the numbers of blood vessels affected and the genotype distribution**

The relation between the number of coronary arteries affected and the genotype distribution in patients with severe coronary stenosis is illustrated in Table 5. The results showed no significant association between the numbers of vessels and the genotype distribution of rs10757278, rs10757274 and rs2383207 polymorphisms.

**Genotype distribution in patients with and without diabetes**

There is no significant difference in genotype distribution of the variants; rs10757278 and rs10757274 in CAD patients with and without DM [Table 6]. In contrast, CAD patients with DM had higher frequency of AG genotype (40.7% vs. 23.1%;  $P = 0.007$ ) than CAD without DM and the overall genotype distribution was also significant for the variant rs2383207.

**DISCUSSION**

The present study evaluates the association of CAD and its severity with four genetic variants in chromosomal locus 9p21 among Arab population residing in Qatar. The interesting finding of this study is the significant association of G allele carriers of rs2383207 SNP with the risk of CAD and its severity. Moreover, subjects having DM and GG carriers for rs2383207 had three-fold increased risk of CAD.

Our findings are consistent with GWAS and other case-control studies that showed an association of genetic polymorphism in chromosomal locus 9p21 and CAD across many ethnic populations.<sup>[7,8,11,12,24-27]</sup> McPherson *et al.* reported two susceptibility SNPs (rs10757274 and rs2383206) that were located within 20 kb of each other on chromosome 9p21 and were associated with CAD among White cohorts.<sup>[6]</sup> Helgadottir *et al.* found that around 21% of subjects belonged to European descent are homozygous for rs10757278-G and their estimated risk of having MI was 1.64-fold higher

**Table 4: Severity angiographic lesions utilizing the recessive and dominant genetic models**

SNP variant	Genetic model	OR (95% CI)	P	AOR (95% CI)	P
rs10757278	AA/AG+GG	0.95 (0.52-1.73)	0.86	1.03 (0.55-1.93)	0.92
	GG/AA+AG	1.56 (0.88-2.77)	0.12	1.60 (0.88-2.91)	0.21
rs10757274	AA/AG+GG	0.69 (0.35-1.56)	0.28	0.69 (0.35-1.36)	0.28
	GG/AA+AG	1.34 (0.79-2.29)	0.27	1.56 (0.88-2.77)	0.12
rs2383207	AA/AG+GG	0.81 (0.26-2.48)	0.70	0.72 (0.36-1.44)	0.34
	GG/AA+AG	1.80 (1.04-3.12)	0.03	1.74 (1.11-3.21)	0.02

AOR was calculated with the following covariates; age, gender, BMI, diabetes, dyslipidemia, hypertension, smoking and family history. Two-tailed  $P$  value is significant <0.05. SNP = Single nucleotide polymorphisms; OR = Odds ratio; CI = Confidence intervals; AOR = Adjusted odds ratio; BMI = Body mass index

**Table 5: Genotype distribution in CAD patients with severe stenosis based on number of affected coronary vessels**

SNP variant	Genotype	1-vessel	2-vessel	3-vessel	P
rs10757278	A/A	16.0 (32.0)	10.0 (19.2)	8 (22.9)	0.268
	A/G	20.0 (40.0)	32.0 (61.5)	17 (48.6)	
	G/G	14.0 (28.0)	10.0 (19.2)	10.0 (28.6)	
rs10757274	G/G	19.0 (37.3)	17.0 (32.7)	14 (40.0)	0.473
	A/G	21.0 (41.2)	29.0 (55.8)	17.0 (48.6)	
	A/A	11.0 (21.6)	6.0 (11.5)	4.0 (11.4)	
rs2383207	G/G	35.0 (60.8)	26.0 (50.0)	20.0 (58.8)	0.063
	A/G	15.0 (29.4)	24.0 (46.2)	14.0 (41.2)	
	A/A	5.0 (9.8)	2.0 (3.8)	0	

Data are presented by numbers and percentage. Data were analyzed by Chi-square test for categorical data. Two-tailed  $P$  value is statistically significant at <0.05. SNP = Single nucleotide polymorphisms; CAD = Coronary artery disease

**Table 6: Genotype distribution in CAD patients with and without diabetes**

Variables	CAD-DM (n = 108)	CAD + DM (n = 128)	P
rs10757278			
AA	31 (28.7)	30 (23.4)	0.623
AG	48 (44.4)	59 (46.1)	
GG	29 (26.9)	39 (30.5)	
rs10757274			
AA	22 (20.4)	21 (16.4)	0.643
AG	47 (43.5)	54 (42.2)	
GG	39 (36.1)	53 (41.4)	
rs2383207			
AA	6 (5.6)	9 (7.0)	0.007
AG	25 (23.1)	51 (40.7)	
GG	77 (71.3)	66 (52.3)	
Numbers of vessels ≥2	31 (53.4)	55 (80.9)	0.047

Data are presented by numbers and percentage. Data were analyzed by Chi-square test for categorical data. Two-tailed  $P$  value is statistically significant at <0.05. CAD = Coronary artery disease; DM = Diabetes mellitus

than the noncarriers.<sup>[7]</sup> To establish the credibility of 9p21 locus-specific contribution to the development of CAD within an Asian population, Shen *et al.*<sup>[10]</sup> studied 4 SNPs on chromosome 9p21 associated with CAD (rs10757274 and rs2383206) and MI (rs2383207 and rs10757278). The authors reported a higher cross-race risk for the development of CAD. A recent meta-analysis on the association of

chromosome 9p21 with early-onset CAD demonstrated a protective effect of the disease association for rs2383207. However, the rs2383206, rs10757278 and rs10757274 variants might contribute to the etiology of early-onset CAD.<sup>[27]</sup> In addition, another study by Chen *et al.* showed that G/G carriers at rs2383207 had a higher risk of having CAD than carriers of A/A genotype.<sup>[28]</sup> The current data showed that the other SNPs of the same locus; rs10757278, rs10757274 are not significantly associated with CAD. These findings are in conflict with previous reports that demonstrated a significant association of these polymorphisms with CAD.<sup>[11,25,29]</sup> The discrepancy between our finding and the previous results could be in part due to the difference in sample size, collection, population stratification and ethnic background.

The current findings refer to the association of the significant atherosclerotic angiographic stenotic lesions based on the genetic association of rs2383207 of 9p21 locus. Previous studies demonstrated an association between the 9p21 risk locus with angiographically defined severity, extent, and progression of CAD, suggesting a role for this locus in influencing atherosclerosis and its progression.<sup>[15,30,31]</sup> In addition, DM is significantly associated with 9p21 locus among CAD with severe stenosis; consistent with a previous study demonstrated an association between common variants on 9p21 and mortality in diabetic patients in Dutch population.<sup>[32]</sup> However, the underlying molecular mechanisms are still not well understood. Several studies addressed the association between this genetic locus and the development of abdominal aortic aneurysm, intracranial aneurysm, carotid atherosclerosis and peripheral vascular disease.<sup>[33-35]</sup> In addition, genetic epidemiologic studies showed that 9p21 variants are associated with vascular smooth muscle cell proliferation that has a role in the pathogenesis of atherosclerosis.<sup>[35]</sup>

This study has several limitations; as for selection bias, population stratification, and the small sample size. Other SNPs associated with CAD could be included to explore the associate between genotype and phenotype of cardiovascular diseases among Arab populations.

## CONCLUSION

G allele of rs2483207 is significantly associated with risk of CAD and its severity especially when associated with DM. No significant association of other SNPs has been identified in the current study with CAD or its severity. Further investigations with larger sample size to clarify mechanisms underlying such associations of these SNPs with CAD are needed.

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## AUTHORS' CONTRIBUTION

AAE, study design, data analysis, interpretation, manuscript draft and approval. NMR, study design, data analysis, interpretation, manuscript draft and approval. AA, study design, data interpretation, manuscript draft and approval. FA, data collection, manuscript draft and approval. AF, data collection, manuscript draft and approval. FF, data collection, manuscript draft and approval. FDB, genetic analysis, data interpretation, manuscript draft. SE, genetic analysis, data interpretation, manuscript draft. EA, data collection, manuscript draft and approval. MA, genetic analysis, data interpretation, manuscript draft. HH, study design, data interpretation, manuscript draft and approval. JA; Study design, data interpretation, manuscript draft and approval.

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